Remarks

Reconsideration and withdrawal of the rejections set forth in the Office Action dated June 13, 2002 are respectfully requested. A separate petition for a 1-month extension of time accompanies this amendment.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

I. Amendments

Claims 1 and 2 are amended to clarify that the virus is purified by physical means without chemical means. Support for this amendment can be found on page 22, lines 21-23 where the inactivated virus is purified by sucrose density gradient zonal ultracentrifugation. Further exemplary methods of purification by physical means without chemical means are presented on page 11, line 29 through page 12, line 2.

Accordingly, no new matter is added by these amendments.

II. Rejections under 35 U.S.C. § 102

Claims 1-3, 9, 15, 17 and 18 were rejected under 35 U.S.C. §102(a) as allegedly anticipated by Ding *et al.* (Zhonghua &I Za Zhi *Natl. Med. J. China* 78:261-262, 1998, hereinafter "Ding *et al.*")

A. The Present Invention

The present invention relates to an inactivated virus particle, as a reinforced immunogen, prepared from a culture of cells infected with virus belonging to a group of Japanese encephalitis viruses. The process of preparation comprises a step of inactivation followed by a step of purification by physical means without chemical means wherein a neutralizing antibody titer of the anti-serum obtained by immunization with the virus particles is about twice to about 10 times the neutralizing antibody titer of the anti-serum obtained by immunization with inactivated virus particles prepared from virus cultured in mouse brain. The effect of the providing the step of inactivation before

the step of purification and that the purification is by physical means without chemical means viruses can be purified in a state such that the surface of the virus is not changed and the antigen is provided in the correct steric conformation for the antigen to react well with the antibodies.

B. The Prior Art

DING *ET AL.* teaches the production of purified Japanese encephalitis vaccine from Vero cells. The virus of Ding *et al* was inactivated, concentrated, treated with protamine sulfate and purified. However, treatment with protamine sulfate is a chemical purification means. The purification of the claimed invention is characterized by purification by physical means without chemical means.

C. Analysis

According to the M.P.E.P. § 2131, "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference".

Ding et al. fails to teach a method for producing an inactivated virus particle or the particle produced wherein the step of inactivation is conducted before purification, and that the purification is conducted by physical means without chemical means.

According to the *Proceedings of the Society for Experimental Biology and Medicine*, 1949 (enclosed herewith), treatment with protamine sulfate is a conventional purification methodology for some viruses including Japanese encephalitis virus (see summary on page 664, col. 2). According to *Comments of the Japanese Pharmacopoeia*, 12th revision, 1991 (enclosed herewith), protamine is a protein which has relatively low molecular weight (10,000 or less) and is strongly basic (an isoelectric point of pH 10-12). It has strongly basic properties due to containing hardly any acidic amino acids, sulfur-containing amino acids, aromatic amino acids and the like. Protamine is such a basic protein that it binds to acidic macromolecules such as nucleic acid and heparin, and can bind to general proteins which have a neutral or slightly acidic isoelectric point so as to precipitate them. Protamine sulfate treatment utilizes

such a precipitation reaction, so as to dissociate virus particle from media and proteins derived from cells.

As noted above, Ding *et al.* teach purification of the Japanese encephalitis vaccine by chemical means using protamine sulfate. Ding *et al.* fail to teach purification which is conducted by physical means without chemical means.

Accordingly, Applicants submit that standard of strict identity to maintain a rejection under 35 U.S.C. § 102 has not been met. Withdrawal of the rejection under 35 U.S.C. § 102(a) is respectfully requested.

III. Rejections under 35 U.S.C. § 103

Claims 1-3, 5, 7, 9-10, 13 and 15-18 are rejected under 35 U.S.C. §103(a) as being obvious over Ding *et al* or Huiying *et al* (1998) in view of Lau *et al.*

A. The Invention

The present invention is described above.

B. The Prior Art

DING *ET AL* is described above.

HUIYING *ET AL.* relates to a method for large scale purification of Japanese Encephalitis vaccine in vero cells. The JE vaccine are concentrated by ultrafiltration and precipitated by protamine sulfate. The vero cells are then purified by zonal centrifugation at non-continuous sucrose gradients.

LAU *ET AL* disclose a process for large-scale purification of living Japanese encephalitis virus from JEV-infected mouse brains and cell cultures wherein formalin inactivation occurs at 4° C. The virus is purified by the steps of microfiltration, ultrafiltration, or gel filtration. Liau *et al.* further describes preparation of a vaccine by purifying the living JEV virus as above, and then inactivating the virus with an inactivating agent such as formalin or binary ethyleneimide.

C. Analysis

According to the MPEP § 2143, "to establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Third, the prior art references (or references when combined) must teach or suggest all the claim limitations."

As noted above, Ding *et al.* fails to teach virus purification which is conducted by physical means without chemical means. The teachings in Huiying *et al.* and/or Liau *et al.* when combined with Ding *et al.* do not make up for this deficiency. Huiying *et al.* disclose the purification of viruses with protamine sulfate. Lau et al. disclose the use of an inactivating agent such as formalin or binary ethyleneimide. Treatment with protamine sulfate, formalin, and binary ethyleneimide are all chemical purification means. Therefore, none of these cited references teach or suggest the feature of the claimed invention which is characterized by physical means without using chemical means.

Accordingly, Applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

IV. Conclusion

In view of the foregoing, the claims pending in the application patentably define over the cited art. A Notice of Allowance is, therefore, respectfully requested. If the Examiner has any questions or believes a telephone conference would expedite prosecution of this application, the Examiner is encouraged to call the undersigned at (650) 838-4410.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

1. (Twice Amended) An inactivated virus particle, as a reinforced immunogen, prepared from a culture of cells infected with virus belonging to a group of Japanese encephalitis viruses, wherein the process of preparation comprises a step of inactivation folowed by a step of purification by physical means without chemical means, wherein a neutralizing antibody titer of the anti-serum obtained by immunization with the virus particles is about twice to about 10 times the neutralizing antibody titer of the anti-serum obtained by immunization with inactivated virus particles prepared from virus cultured in mouse brain.

2. (Twice Amended) A method for producing an inactivated virus particle, comprising culturing virus belonging to a group of Japanese encephalitis viruses in a cell line, inactivating the cell culture and then purifying the virus by physical means without chemical means, wherein a neutralizing antibody titer of the anti-serum obtained by immunization with the virus particles is about twice to about 10 times the neutralizing antibody titer of the anti-serum obtained by immunization with inactivated virus particles prepared from virus cultured in mouse brain.